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Brief Research Communication

Neuronal Nicotinic Acetylcholine Receptor α4 Subunit (CHRNA4) and Panic Disorder: An Association Study

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Anxiety disorders have been reported to be associated with low-voltage EEG (LVEEG). Some cases with LVEEG (approximately ⅓) have been linked to chromosome 20q13.2–q13.3. In the same chromosomal region, the gene for the neuronal nicotinic acetylcholine receptor α4 subunit (CHRNA4) has been located. We therefore tested the hypothesis that polymorphisms in the CHRNA4 gene show an allelic association with panic disorder. We examined the allele frequencies of three different CHRNA4 polymorphisms in patients with panic disorder and in healthy controls. No significant differences in the allele frequencies of these three polymorphisms were noted. This study does not support an association between panic disorder and the CHRNA4 gene. Am. J. Med. Genet. 74:199–201, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: nicotinic acetylcholine receptor; panic disorder; association

INTRODUCTION

Panic disorder is characterized by recurrent spontaneous anxiety attacks with numerous somatic complaints leading to severe impairment of the patient. It has a lifetime prevalence of about 1–3%, typically begins in the midtwenties, and is more common among women than men [Eaton et al., 1994; Robins and Regier, 1991; Whitaker et al., 1990]. Panic disorder frequently takes a chronic course with many remissions and relapses, occasionally complicated by comorbidity with other psychiatric disorders such as depression or alcohol abuse [Goldstein et al., 1994; Schuckit and Hesselbrock, 1994; Keller and Hanks, 1993]. It is generally accepted that panic disorder has genetic as well as environmental causes. The genetic factors have been demonstrated by family as well as twin studies. [Crowe et al., 1983; Maier et al., 1993; Skre et al., 1993].

Panic disorder, as well as other types of anxiety disorders and alcoholism combined with anxiety disorder, were recently found to be significantly associated with the low-voltage electroencephalogram trait (LVEEG) [Enoch et al., 1995]. LVEEG is a variant of the normal human resting EEG, lacking rhythmical alpha activity. It usually shows a low overall EEG amplitude, with short segments of alpha rhythm appearing occasionally for 1–2 sec after closure of the eyes, or after provocation tests such as hyperventilation or intermittent photic stimulation in the alpha frequency range [Anokhin et al., 1992]. The mode of inheritance is autosomal dominant, with incomplete penetrance in younger persons [Vogel and Götze, 1959; Vogel, 1962; Anokhin et al., 1992]. The reported rates of prevalence vary between 3–5% [Gibbs et al, 1943; Vogel and Götze, 1959; Vogel and Fujiya, 1969]. A gene responsible for about one third of cases of low-voltage EEG (EEGV1) was genetically mapped to 20q13.2–q13.3, with maximum likelihood to the polymorphic locus CMM6 (D20S19) [Steinlein et al., 1992a].

The neuronal nicotinic acetylcholine receptor α4 subunit (CHRNA4) was localized to a chromosomal segment in the vicinity of CMM6 (D20S19), between the polymorphic loci RMR6 (D20S20) and IP20K09 (D20S24) [Steinlein et al., 1992b, 1994]. Thus, CHRNA4 is a possible candidate gene for disorders localized to this chromosomal region. In a large Australian family, a serine-to-phenylalanine exchange in amino-acid position 248 of the CHRNA4 gene was shown to be present in autosomal-dominant nocturnal frontal lobe epilepsy [Steinlein et al., 1995].

Since low-voltage EEG was reported to be common in individuals with anxiety disorders, we hypothesized that CHRNA4 variants might be associated with panic

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disorder. Panic disorder patients (n = 88) were in- as well as outpatients of German descent from cooperating psychiatric university departments or private practices. Panic disorder was diagnosed according to DSMIII-R [American Psychiatric Association, 1987] by taking into account first medical records, and second a structured clinical interview (n = 40 probands with SADS-LA, n = 48 probands with CIDI). The final diagnosis for the consensus diagnosis was assessed by an experienced psychiatrist. Only patients with predominant panic disorder, which occurred primarily in the course of the disorder, were included. Informed consent was obtained. Patients showed the typical clinical features of panic disorder. There was a greater proportion of females than males (ratio, about 2:1). Comorbidity in decreasing frequency was observed for phobias, depressive disorders, and alcohol dependence (Table I). Panic disorder could be diagnosed in first-degree relatives of 7 patients, indicating that at least 8% of patients may have represented familial rather than sporadic cases. Controls were randomly chosen from a panel of anonymous blood donors of German descent.

In addition to the *Cfo*I polymorphism at bp 594 of the CHNRNA4 gene described earlier [Steinlein, 1995], two newly detected same-sense polymorphisms were used in this study: a G-to-A nucleotide exchange in intron 2 (bp 145^{-55}; base-pair numbering according to Steinlein et al. [1996]) abolishes in *Hae*III site; a T-to-C exchange in exon 5 (bp 1545) creates a *Cfo*I site. The three polymorphisms were equally spaced over the coding region of CHRNA4. For the *Hae*III polymorphism, a 148-bp genomic DNA fragment was amplified using primers 5′-CCCGTCCACCATATCTTGC-3′ and 5′-GGCAGTGCCCTCCCACTC-3′ with 32 cycles and an annealing temperature of 60°C. *Hae*III digestion of the amplified fragment showed the following band pattern on a 10% nondenaturing polyacrylamide gel: 22 bp, 42 bp, 28 bp, and 92 bp (G-allele); and 22 bp, 70 bp, and 92 bp (A-allele). For the *Cfo*I polymorphism at bp 1545, the following primers were used: 5′-ACCAGGGCTG-GCCAAAGCCAGG-3′ and 5′-GTGCTTTGGTGCTGC-GGGTC-3′. Amplification was carried out for 32 cycles with an annealing temperature of 64°C. *Cfo*I digestion of the PCR product showed the following band pattern: 152 bp and 138 bp (T-allele); and 152 bp, 105 bp, and 33 bp (C-allele) (Fig. 1). Each PCR was carried out in a 50μl volume containing 80 ng of genomic DNA, 200 mM of each dNTP, 20 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 5% glycerin, 5 pmol of each primer, and 1.7 units Taq-Polymerase (Pharmacia).

TABLE I. Clinical Characteristics of Patients With Panic Disorder (n = 88)*

Sex (M/F)	31/57	(35.2%/64.8%)
Age (years)	38.9 + 11.0	(21–77)
With agoraphobia	61	(69.3%)
Comorbidity		
Simple phobia	39	(44.3%)
Social phobia	28	(31.8%)
Major depression	23	(26.1%)
Dysthymia	16	(18.2%)
Alcohol dependence	13	(14.8%)

*Patients were diagnosed on the basis of clinical records and standardized interviews (SADS-LA or CIDI) according to DSMIII-R. Only diagnoses obtained in more than 10% of patients are listed.

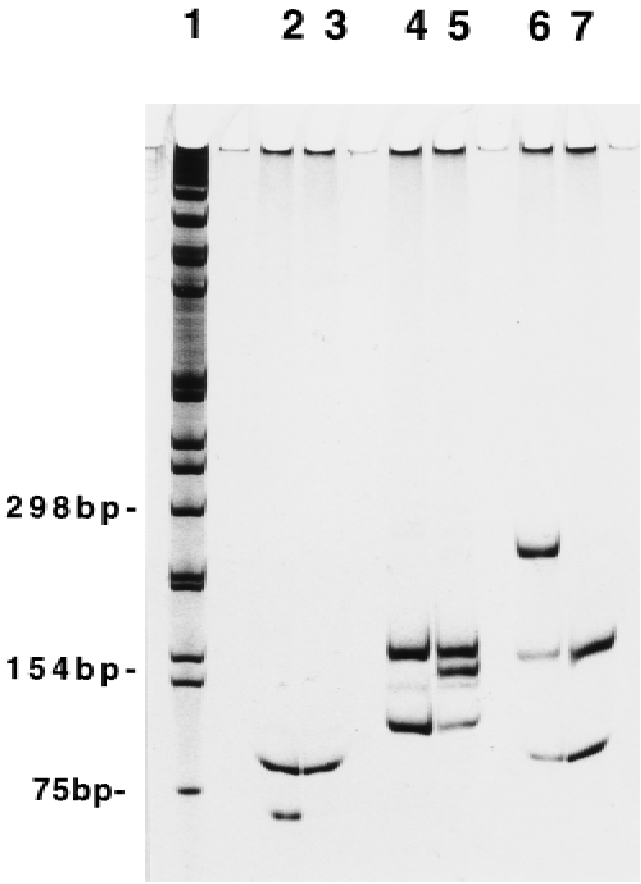

Fig. 1. Restriction analysis of CHRNA4 polymorphisms. **Lane 1,** DNA standard (1-kb ladder, Gibco BRL); **lanes 2–3,** *Hae*III polymorphism at bp 145^{-55}, G/A-allele heterozygosity (lane 2), G-allele homozygosity (lane 3); **lanes 4–5,** *Cfo*I polymorphism at bp 1545, C-allele homozygosity (lane 4), C/T-allele heterozygosity (lane 5); **lanes 6–7,** *Cfo*I polymorphism at bp 594, C/T-allele heterozygosity (lane 6), C-allele homozygosity (lane 7). Fragments smaller than 50 bp are not shown.

The heterozygotes and homozygotes for each polymorphism were counted and compared with frequencies expected under the Hardy-Weinberg equilibrium. The χ^2 test for 2 × 2 tables was used to test for allelic association of CHRNA4 polymorphisms with panic disorder. All genotype frequencies were within Hardy-Weinberg expectations. Heterozygosity rates were 0.12, 0.11, and 0.48 for bp 145^{-55}, bp 594, and bp 1545 polymorphisms, respectively. No linkage disequilibrium was observed between the three polymorphisms.

The allele frequencies of the three silent polymorphisms in the patients did not differ significantly from the allele frequencies observed in the control group (Table II). Our findings therefore do not support the possibility of an association between CHRNA4 and the liability to develop panic disorder, and argue against a major role for a single mutation in the CHRNA4 gene for panic disorder. A possible explanation for the failure to find an association between CHRNA4 and panic disorder may be that only about one third of all LVEEG phenotypes are due to a gene residing on chromosome 20q. Enoch et al. [1995] assumed a population fre-

TABLE II. Allele Frequencies in Patients and Controls*

CHRNA4 polymorphism	Panic disorder	Controls
bp 145^{-55}/*Hae*III		
A-allele	0.062	0.068
G-allele	0.938	0.932
	(n = 176)	(n = 176)
bp 594/*Cfo*I		
T-allele	0.059	0.027
C-allele	0.941	0.973
	(n = 168)	(n = 184)
bp 1545/*Cfo*I		
T-allele	0.542	0.528
C-allele	0.458	0.472
	(n = 166)	(n = 162)

*No statistically significant difference in allele frequency was observed ($P > 0.05$, chi-square analysis). n, number of chromosomes.

quency of 10% for LVEEG, which leads to the relative risk of approximately 9 for the group of patients with panic disorder or the group including the patients with either general anxiety or panic disorder. Relative risks are considerably higher if the population frequency of approximately 5% for LVEEG, given by a representative study by Vogel and Fujiya [1969], is taken. For example, the A-allele of the bp 145^{-55}/*Hae*III polymorphism and the T-allele of the bp 594/*Cfo*I polymorphism were estimated to have a power of approximately 0.82 to detect a relative risk of 3, and of approximately 0.81 for a relative risk of 4, respectively. This means that, even if only 30% of LVEEGs were caused by a gene on chromosome 20q, the bp 145^{-55}/*Hae*III and bp 594/*Cfo*I polymorphisms had enough power to detect possible associations, assuming that the unknown mutation occurred close to the rare allele.

Negative findings in association studies do not necessarily exclude a possible pathogenetic role of the gene under study. The possibility remains that mutations exist in the CHRNA4 gene which are not in linkage disequilibrium with the examined polymorphisms. Other polymorphisms have to be found in the region and subsequently tested in samples of patients with panic disorder or other anxiety disorders. Furthermore, direct mutation screening efforts in other candidate genes located in this region should be envisaged.

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